3.7.2 Collection, Processing, and Shipping of Blood Samples

I. PURPOSE

1. The Central Blood Analysis Laboratory (CBAL) will be responsible for special blood collection and handling protocols as well as training and QC monitoring at the Clinical Centers. The laboratory will also be responsible for performing assays and reporting results.

2. The blood samples (65.5 mL from participants at enrollment) that are collected and processed by Clinical Center technicians are the foundation for all of these tests. The most important step—and potentially the most variable—is the collection and processing of the blood samples. If samples are not correctly drawn and processed, the laboratory results may not be precise or valid.

II. EQUIPMENT & SUPPLIES

1. CBAL will provide the following supplies in bulk:

   - 5 mL SCAT-I tubes (must be refrigerated until use)
   - 8 mL cell preparation tubes (CPT)
   - 15 mL centrifuge tubes
   - 0.5, 2.0, 4.0, and 10.0 mL cryovials and tubes, with colored caps for coding
   - Dextran preservative (ACD solution)
   - Freezing Media A (must be stored at -20 C°)
   - Freezing Media B (must be stored at -20 C°)
   - PBS tablets

2. The blood collection area should have the following supplies:

   - Lab coats and gloves
   - Phlebotomy chair
   - Plastic cart with wheels (or plastic tray with compartments) for supplies
   - Ice bucket with crushed ice, filled 10 minutes before blood draw
   - Blood tube racks
   - Blood tube rocker
   - Basin (just in case)
   - Smelling salts
   - Washcloths and towels
   - Lab mats and wipes
   - Participant ID labels
   - Phlebotomy Form
   - MESA Processing Form

- Pens
- Blood collection tubes:
  - two 10 mL EDTA tubes (BD 366457)
  - two 10 mL red-topped serum tubes (BD 366430)
  - one 4.5 mL citrate tubes (BD 366415)
  - one 5 mL SCAT-I (provided by CBAL)
  - two 8 mL CPT (provided by CBAL)
- For Blind Duplicate Samples: 5 mL serum (B-D 366534) and 5 mL EDTA (B-D 366452) tubes
- Alcohol prep pads
- Tourniquets (quick-release tourniquet, supplied by the blood lab, is recommended; please do not use blood pressure cuff)
- 21 gauge Butterfly needles with luer adapter (BD #7251)
- Vacutainer barrels
- Timer/stopwatch
- Scissors
- Surgical tape/paper tape
- "Bandaids"
- Gauze (2x2-inch)
- Blood spill kit
- Biohazards waste container
- Needle/sharps container
- 10% bleach solution or approved biohazard disinfectant

III. DEFINITIONS

IV. METHODS

1. Safety Issues and Precautions for Handling Blood Specimens.

   In accordance with the Occupational Safety and Health Administration (OSHA) regulations on bloodborne pathogens, the CBAL recommends the following laboratory safety protocol for the field center laboratories:

   1.1 Use non-permeable lab coats, latex gloves, and face shields when handling any blood in any situation in which splashes, spray, spatter, or droplets of blood may be generated and eye, nose, or mouth contamination can be reasonably anticipated.

   1.2 Use aerosol containers in all centrifuges.

   1.3 Follow 'Universal Precautions' when handling any blood products.

   1.4 Immediately place contaminated needles and sharps in a puncture-resistant, leak-proof container. Never recap or break needles.

1.5 Offer Hepatitis B vaccine to all unvaccinated technicians who handle blood. Documentation of vaccination, or technician's refusal to be vaccinated, should be kept on file at the Clinical Center.

2. Participant ID Labels

2.1 The Coordinating Center will supply each field center with sheets of sample ID barcode labels to use for labeling draw tubes, working tubes, cryovials, and freezer boxes. There will be a total of 80 labels (some of which will be used for urine collection and processing):

- 8 labels for the draw tubes
- 1 label for the freezer box
- 65 cryovial labels
- 6 extra labels

2.2 Each set of participant barcode labels has the same 7-digit sample identification number. (The first digit identifies the clinic. Wake Forest is 3, Columbia 4, John's Hopkins 5, University of Minnesota 6, Northwestern 7, UCLA 8.) The cryovial labels also have a 2-digit extension (01 to 62) that uniquely identifies each cryovial within a sample ID and helps in tracking the repository. See Appendix for proper orientation of the barcode label on the cryovial.

2.3 There will also be special QC ID labels for the blind duplicate samples. See section 6.7 for further information about this procedure.

2.4 Blood samples must be precisely labeled throughout the collection and processing stages to ensure they are correctly coded. Always pre-label sets of collection tubes and cryovials prior to the participant's visit, and cross-check the labels with each participant's ID number prior to the phlebotomy.

3. Forms

3.1 The Phlebotomy Form and MESA Processing Form provide a vital link between the sample ID number and the participant ID number and facilitate the efficient collection of plasma and serum samples. In addition, the Phlebotomy Form facilitates the monitoring of phlebotomy and other quality assurance parameters and provides information critical to the interpretation of the assay results.

3.2 The Phlebotomy Form will be scanned, and the information will be electronically sent to the Coordinating Center and the CBAL. The completed Phlebotomy Form and MESA Processing Form will then be

sent with the sample shipments to CBAL. Both forms must be labeled with the correct pre-printed barcode sample ID label. All forms must be completed in ink.

3.3 Please note: Until further notice, continue to include the Phlebotomy Form in the sample shipment.

4. Participant Refusal of Phlebotomy

Rarely, a participant will refuse phlebotomy. Please keep a list of MESA Enrollment ID numbers of any of these participants and identify which test they refused.

5. Venipuncture

5.1 Initial preparation for specimen collection. Prior to the arrival of participants:

- Make sure venipuncture supplies are stocked. Have kits ready. Make sure tubes and cryovials are labeled.
- Make sure the sample processing station is properly equipped. Every item on the checklist must be ready and in its proper position.
- Make sure the phlebotomy room is tidy and stocked with extra smelling salts, basins, and wash cloths, and that the draw tube mixer is working.
- Label the tubes and cryovials with the participant ID.
- Approximately 10 minutes before scheduled blood specimen collection, fill Styrofoam ice bath 3/4 full with crushed ice.

5.2 Preparation of draw tubes and aliquot racks

To facilitate accurate tracking of collected specimens, set up a blood collection tube rack with the set of draw tubes that are pre-labeled with the provided participant ID labels. The tubes should be in the rack according to the order in which they are to be drawn, as specified in section 5.3, below. Set up an aliquot rack, with pre-labeled cryovials, to correspond with each participant's blood collection tube rack. It may be helpful to have the red cryovials in a separate rack, because the red serum collection tubes are generally centrifuged at a different time from the other tubes.

5.3 Priority of tubes

Approximately 65.5 mL of blood will be drawn from each participant and collected into eight tubes. The order in which the tubes are collected is *extremely important* and must be done as follows:

1. 10 mL EDTA (purple top)
2. 10 mL serum (red top)
3. 8 mL cell prep tube (CPT) (blue & black top)
4. 5 mL special coagulation tube (SCAT-I) (red top)
5. 4.5 mL citrate (blue top)
6. 10 mL EDTA (purple top)
7. 10 mL serum (red top)
8. 8 mL cell prep tube (CPT) (blue & black top)

5.4 Collection of Blind Duplicate Tube

Twenty percent of participants will have an additional tube of blood collected, for a total of nine tubes/approximately 70 mL of blood. This sample is collected for quality control purposes. This sample is collected *last* into the Blind Duplicate Tube (#9), which may be purple, red, SCAT, or blue.

5.5 Preparation of Phlebotomy Room

The blood draw is done in an isolated room, or participants are separated by room dividers. The room is equipped with all of the necessary blood drawing supplies. A separate counter or work table is equipped with all of the materials and vials that are used in the blood handling and processing. The centrifuge, refrigerator, and freezer should be nearby.

5.6 Preparation of Participants

5.61 Ensure that informed consent has been obtained before drawing blood. This study depends on and requires the *voluntary* cooperation of the participants. These people are *giving* their time—and precious bodily fluids—and their only reward is the knowledge that they are contributing to progress in medicine. Thus, the experience must be as pleasant as possible. Give the participant enough time to feel comfortable, both before and after the blood collection. In many cases the most memorable part of the experience for the participant will be the contact with, and the attitude and competence of, the technician who draws the blood. Do *not* under any circumstances force or coerce the participant to have blood drawn.

5.62 Eight (or nine—see 5.4, above) tubes of blood of various sizes are collected, each containing about 1–2 teaspoons (5–10 ml) of blood. Participants who are concerned about the volume of blood should be reassured that the total amount of blood drawn is about

5 tablespoons, although it may look like more. The phlebotomist may also assure participants that more than six times as much blood (450 ml) is collected when they donate a unit of blood.

5.7 Venipuncture Procedure

ALWAYS WEAR LATEX GLOVES AND LAB COAT

Blood drawing is standardized for the sitting position. You may have participants clench their fists (moderately) during phlebotomy, for up to two minutes. Venipuncture is performed with a 21-gauge butterfly needle with 12 inches of plastic tubing between the venipuncture site and the blood collection tubes. The butterfly has a small, thin walled needle that minimizes trauma to the skin and vein. Using 12 inches of tubing allows tubes to be changed without any movement of the needle in the vein. It also allows the collection of non-sterile SCAT-I tubes by eliminating the possibility of blood back-washing from tube to participant. Step-by-step procedures are as follows:

1. Arrange draw tubes in order of draw on the table top or in the tube rack within easy reach. Assemble butterfly apparatus and vacutainer holders, gauze, and alcohol prep prior to tourniquet application.

2. Apply tourniquet (quick-release tourniquet, supplied by the blood lab, is recommended; please do not use a blood pressure cuff).

3. Examine participant's arms for the best site for venipuncture. Release tourniquet.

4. Cleanse venipuncture site by wiping with alcohol prep pad in a circular motion from center to periphery. Allow area to dry.

5. Re-apply tourniquet and start timer. Document start time. (It is best to *release the tourniquet as soon as possible* after flow has been established. The tightened tourniquet should be on no longer than two minutes; if it is necessary to have it on longer than two minutes, loosen the tourniquet and then re-apply. However, this may result in cessation of blood flow, especially in sick and/or elderly participants, and may result in the need for a second venipuncture.)

6. Grasp the participant's arm firmly, using your thumb to draw the skin taut to anchor the vein. The thumb should be one or two inches below the venipuncture site.

7. With the needle bevel upward, enter the vein in a smooth

continuous motion.

8. Make sure the participant's arm is in a flat or downward position while maintaining the tube below the site when the needle is in the vein. It may be helpful to have the participant make a fist with the opposite hand and place it under the elbow for support.

9. Grasp the flange of the vacutainer holder and gently push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle. (*Minimize turbulence* whenever possible. Small steps, such as slanting the needle in the vacutainer to have the blood run down the side of the tube instead of shooting all the way to the bottom, may result in significant improvement.)

10. Note the blood flow into the first collection tube. If blood is flowing freely, the butterfly needle can be taped to the participant's arm for the duration of the draw. If the flow rate is very slow, the needle may not be positioned correctly. Try moving the needle slightly without causing discomfort to the participant.

11. If the collection tube does not fill, try another tube of the same type. (Partially-filled plasma tubes are not acceptable if less than two-thirds full. Partially-filled serum tubes are okay but will result in a reduced number of aliquots. If a tube is not completely filled, clearly note on Phlebotomy Form.)

12. Keep a constant, slight forward pressure (in the direction of the needle) on the end of the tube. This prevents release of the shut-off valve and cessation of blood flow. Do not vary pressure or reintroduce pressure after completion of the draw.

13. Fill each vacutainer until the vacuum is exhausted and blood flow ceases. If a vacutainer tube fills only partially, remove the tube and attach another without removing the needle from vein. Tubes <½ full are not acceptable

14. When the blood flow ceases, remove the tube from the vacutainer holder. The shut-off valve re-covers the point and stops blood flow until the next tube is inserted (if necessary).

15. Release tourniquet, if still applied.

16. To remove the needle, lightly place clean gauze over venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Have the participant hold the gauze

pad firmly for one to two minutes to prevent formation of a hematoma. Discard needle into puncture-proof sharps container. Record on Phlebotomy Form the duration the tourniquet was applied and length of venipuncture.

17. Place all tubes, except serum and CPT, on the tube mixer for a minimum of 30 seconds. CPTs are to be *gently* inverted several times after being drawn.

18. If the participant continues to bleed, apply pressure to the site with a gauze pad. Keep the arm elevated until the bleeding stops. If necessary, tightly wrap a gauze bandage around the pad and leave in place for at least 15 minutes.

19. Place the citrate, EDTA, and SCAT-I tubes on wet ice. Hold the serum and CPT tubes at room temperature.

20. Clean up the venipuncture area (if necessary). Dispose of needle and tubing in the appropriate biohazard needle sharps containers. Complete the Phlebotomy Form.

20. Take the filled blood collection tubes to the processing area, keeping the EDTA, citrate and SCAT-I tubes on ice and the serum and CPT tubes at room temperature.

5.8 Summary of Blood Mixing During Venipuncture

- Tubes 1, 6, and 9 (EDTA): place on mixer for ~30 seconds, then place in ice bath.
- Tubes 3 and 8 (CPT): *gently* invert several times before placing in rack at room temperature; process within 3 hours.
- Tube 4 (SCAT-I): place on mixer for *at least* 30 seconds, then place in ice bath.
- Tube 5 (Citrate): place on mixer for ~30 seconds, then place in ice bath.
- Tube 2 and 7 (serum): do *not* mix; place in rack at room temperature for *at least* 40 minutes.

5.9 Guidelines for Difficulties

5.91 Assisting participants who are extremely apprehensive about giving blood. Explain to the participant that the blood draw is designed to be as painless as possible. Have the participant relax in the blood drawing chair just so the phlebotomist can check the veins in the participant's arms, without actually drawing blood. If the participant has "good veins," reassuringly say, "Oh, you have

good veins; there should be no problem." It may help to let the participant go on with another part of the visit and return later for the blood draw. Do not, under any circumstances, force the participant to have blood drawn.

5.92 Procedures for a difficult draw. If a blood sample is not forthcoming, the following manipulations may be helpful:

- If there is a sucking sound, turn needle slightly or lift the holder in an effort to move the bevel away from the wall of the vein.
- If no blood appears, move needle slightly in hope of entering vein. Do not probe. If not successful, release tourniquet and remove needle. A second attempt can be made on the other arm.
- Loosen the tourniquet. It may have been applied too tightly, thereby stopping the blood flow. Reapply the tourniquet loosely. If the tourniquet is a Velcro type, quickly release and press back together. Be sure, however, that the tourniquet remains on for no longer than two minutes at a time.
- Do not attempt a venipuncture more than twice.
- Reassure the participants that your inability to obtain a clean venipuncture is not any sign of a medical problem on their part.
- If venipuncture is unsuccessful, note on the Phlebotomy Form.

5.93 Assisting participants who look or feel faint.

- Have the participant remain in the chair and sit, if necessary, with head between knees until his/her color returns and he/she feels better.
- Provide a basin if the participant feels nauseated.
- Place a cold wet cloth on the back of the neck.
- If the participant faints, use smelling salts to revive by crushing the ampoule and waving it under the nose for a few seconds.
- If the person continues to feel ill, contact a medical staff member for advice.

5.94 If a collection tube does not fill, try another tube of the same type. Partially-filled plasma tubes are not acceptable, and should be discarded, if less than ½ full. Serum tubes less than ½ full are acceptable but will yield a reduced number of aliquots. If the tube is not completely filled, note this on the Processing Form, as this can affect future assays

5.95 If all tubes are not collected (blood flow ceases, difficult venipuncture, etc.), make a note of the difficulties on the Phlebotomy/Processing Form. *Always fill collection tubes in the order specified, except as noted, below.* If the participant is willing, another attempt should be made to complete the draw, collecting *only* those tubes that were not filled in the first attempt. If you will be re-starting at tube #4 (SCAT-I), *first collect tube #5,* followed by #'s 4, 6, 7, 8 (and 9, if applicable).

6. Processing Specimens

6.1 Overview

Initiate processing as soon as possible (15–30 minutes) following venipuncture. You *must* wear personal protective equipment (non-permeable lab coats, double-gloves with at least one latex pair, splatter shields) during processing.

6.2 Daily Preparation

The following items should be on hand before beginning to process specimens:

- Lab coat, ample supply of latex gloves, splash shield
- Emergency eye wash station
- Biohazards waste disposal container
- 10% bleach solution or approved biohazard disinfectant
- Freezer (-70°C or colder)
- Refrigerator for storage of special blood tubes, freezing media, and tubes #18 and #19 (may be a household refrigerator but may not be used for food storage)
- Fixed-volume pipettes with tips (MLA) and adjustable pipettes (Rainin, Finn, etc.) with tips. Volumes needed: 230 µL, 0.5 mL, 1.0 mL (200-1000 µL), 3–5 mL, 9 mL, and 14 mL.
- Transfer pipette
- Cryovial/freezer box labels (provided by the CC)
- Cryovial and test tube racks
- Cryovials (0.5 mL, 2.0 mL, 4.0 mL, 10.0 mL) (provided by CBAL)
- 15 mL tubes for pooling sample
- 15 mL centrifuge tubes for cell prep (provided by CBAL
- Freezing media A (provided by CBAL)
- Freezing media B (provided by CBAL)
- Materials for cell prep procedure:
- PBS (phosphate buffered saline) (provided by CBAL)
- distilled water

- graduated cylinder
- reagent bottle
- ACD/dextran solution (for red cell membranes) (provided by CBAL)
- Reagent bottles/containers for daily use
- Labels/lab tape for reagent bottles
- Sharpie pens
- Refrigerated centrifuge: 2000 g-force minimum, 4°C, swinging bucket
- Test tube holder (adapters) for centrifuge
- Harvard Trip balance/Pan balance
- Water bottles for balance
- Revco boxes and dividers (10 x 10 and 7 x 7 grids)
- Styrofoam/insulated shipping boxes
- Nalgene #5100-001 freezing container "Mr. Frosty" (3) (provided by CBAL)
- 100% isopropyl alcohol

6.3 Instructions for centrifuging EDTA, SCAT-I, citrate, and serum tubes

6.31 EDTA, SCAT-I, and citrate tubes (#s 1, 6, 4, and 5, respectively) should be stored upright on wet ice for *no longer than 30 minutes,* if centrifugation cannot be done immediately. Allow serum tubes (#s 2 and 7) to clot for at least 40 minutes (but no longer than 90 minutes) at room temperature. Please note all start times on the Processing Forms.

6.32 Centrifuge tubes at 4° C at at least 2000 g for 15 minutes or 3000 g for 10 minutes, for a total of 30,000 g-minutes. Once centrifugation is complete, carefully place tubes on ice in preparation for pooling and aliquoting.

6.4 Guidelines for aliquoting

6.41 Aliquoting involves removing the serum or plasma in small amounts (e.g., 0.5 mL) by pipette and placing it into the appropriate color-coded cryovials (provided). Color-coding is predetermined and used to identify sample type.

6.42 This process must be done while the tubes and cryovials are on ice (unless otherwise noted).

6.43 When aliquoting serum and plasma, be careful not to disturb the top of the cell pellet with the pipette tip, as this will result in platelet, white cell, and red cell contamination.

6.44 Use a new pipette tip for each draw tube.

6.45 Pool plasma or serum of like tubes from the same participant (e.g., plasma from tubes 1 and 6; serum from tubes 2 and 7).

6.46 If any tubes are accidentally mixed during pipetting, so that plasma is contaminated with red cells, they may be recentrifuged.

6.47 If there is insufficient sample of a tube type to make the full set of aliquots, fill the cryovial that is marked with an asterisk (*) on the Processing Form for that tube type first. Any partially-filled (less than the specified volume) cryovial should be marked with a dot on the cap and a "P" in the comment field of the Processing Form next to that cryovial number.

6.48 Discard tubes after pooling and aliquoting are completed.

6.5 Instructions for aliquoting EDTA, SCAT-I, citrate, and serum tubes

6.51 Description of aliquots

Tubes	Type	Number of Cryovials	Color Code	Volume per Cryovial
1, 6	10 mL EDTA	16 (#s 1–16)	white	0.5 mL
		1 (#17)	white	1.0 mL
		2 (#s 18 & 19)	white	3–5 mL packed red blood cells
4	5 mL SCAT-I	4 (#s 20–23) [1 (#18)]	yellow [white]	0.5 mL [pRBCs]
5	5 mL citrate	4 (#s 24–27) [1 (#19)]	blue [white]	0.5 mL [pRBCs]
2, 7	10 mL serum	17 (#s 28–44)	red	0.5 mL

6.52 <u>EDTA plasma→cryovials 1–17</u>. Pool plasma from tubes #1 and #6 in a 15 ml tube. Aliquot, by volumes specified in the table above, into white-topped cryovials 1–17. Freeze cryovials in an upright position at -70^{o} C.

6.54 <u>EDTA packed red blood cells (pRBCs)→vial 18</u>. Transfer all (usually ~ 3–5 mL) of the EDTA pRBCs from tube #1 to vial 18. Also add the pRBCs from the SCAT-I and citrate tubes to vial 18. *Refrigerate.*

6.55 <u>EDTA packed red blood cells (pRBCs)→vial 19</u>. Transfer all (usually ~ 3–5 mL) of the EDTA pRBCs from tube #6 to vial 19. Carefully add an equal volume of ACD solution (90% dextran preservative). Invert the vial several times to mix. *Refrigerate.*

6.56 SCAT-I→cryovials 20–23. Aliquot 0.5 mL of the plasma from tube #4 into each of yellow-capped cryovials 20–23. The remaining pRBCs are added to cryovial 18 (see 6.54, above). Freeze cryovials in an upright position at -70° C.

6.57 Citrate→cryovials 24–27. Aliquot 0.5 mL of the plasma from tube #5 into each of blue-capped cryovials 24–27. The remaining pRBCs are added to cryovial 18 (see 6.54, above). Freeze cryovials in an upright position at -70° C.

6.58 Serum→cryovials 28–44. Pool and aliquot serum at room temperature. Carefully pipette 0.5 mL of pooled serum into each of red-capped cryovials 28–44. Freeze cryovials in an upright position at -70° C.

6.6 Processing cell preparation tubes (CPT)

6.61 Description of aliquots

Tubes	Type	Number of Cryovials	Color Code	Volume per Cryovial
3, 8	8 mL CPT	16 (#s 45–60) 2 (#s 61 & 62)	green clear	0.5 mL PRP[1] 2.0 mL (cells)

[1] PRP = Platelet Rich Plasma from the citrated CP tube.

6.62 Separation of cells (buffy coat). Tubes #3 and #8 may be held at room temperature for up to three hours to allow batching of CP tubes from several participants. If samples from several participants are run at once, however, it is *very important* to ensure that the samples do not get mixed up. *In addition, for steps 1–4, below, it is* ***absolutely imperative*** *that pipette tips and transfer pipettes be changed between samples from different participants.* Failure to do so could invalidate DNA results.

6.63 Step 1: Centrifugation of CP tubes. Please note that these tubes are taller (13 cm) than other sample collection tubes, so a different rotor may be necessary. Centrifuge CP tubes at *no more than* 2,000 g for 15 minutes, for a total of 30,000 g-minutes, at *room temperature.* This may require a refrigerated centrifuge set at room temperature (approximately 20° C). Do *not* allow the centrifuge to overheat. When centrifugation is complete, several layers will be evident. The top layer is citrated plasma (PRP = platelet rich plasma). Under this is a whitish cell layer (buffy coat) used for cell collection. Next is a gradient gel layer that acts as a barrier to prevent contamination by the red blood cells (RBCs) that are at the bottom of the tube.

6.64 Step 2: Removing Plasma. *In this step, be very careful not to disturb the buffy coat layer. It is OK to leave some extra plasma in the tubes—it will be separated from the buffy coat layer during the next centrifuge step.* Using a transfer pipette, pool the plasma layer from CP tubes #3 and #8 into a 15 mL tube and then aliquot 0.5 mL into each of green-capped cryovials 45–60. Freeze cryovials in an upright position at -70° C

6.65 Step 3: Pelleting the buffy coat. Using a transfer pipette, completely remove the buffy coat layer from CP tube #3 and transfer to a separate 15 mL conical centrifuge tube labeled with the participant's ID number. Fill the centrifuge tube up to the 14 mL mark with phosphate buffered saline (PBS). Mix gently. Repeat this process with the buffy coat layer from tube #8. Centrifuge tubes for 4500 g-minutes at room temperature. (If you need help calculating the centrifuge speed and time appropriate for your centrifuge, contact Elaine Cornell at the CBAL.)

6.66 Step 4: Resuspension of buffy coat pellet. After centrifugation is completed, there should be a small pellet in the bottom of the tube. Using a transfer pipette, carefully remove the PBS solution from the tube. Do not disturb the pellet (if necessary, leave a small amount of PBS solution in the tube). Add 0.5 mL of buffy coat freezing media A to the centrifuge tube containing the buffy coat pellet. Resuspend the pellet by tapping the tube and/or flushing the pipette tip repeatedly. This may take a little patience. When the pellet has been resuspended, add 0.5 mL of buffy coat freezing media B and mix until the pellet is completely dissolved. Using a transfer pipette, transfer the resuspended buffy coat to clear-capped cryovial #61. Repeat this process with the second centrifuge tube, transferring the resuspended buffy coat to cryovial #62. Be sure the cryovial labels match the centrifuge tube labels. Place the cryovials in a rack that can be placed inside a Nalgene "Mr. Frosty" for slow freezing.

6.67 Freezing Steps: "Mr. Frosty" must be at room temperature when cryovials #61 and #62 are placed inside him. The rack holding the cryovials is placed inside "Mr. Frosty," and then the entire container is placed in a -70°C or colder freezer for a minimum of four hours (preferably overnight). Once "Mr. Frosty" goes into the freezer, additional cell cryovials may not be added. When "Mr. Frosty" is removed from the freezer in the morning, remove the now-frozen cell cryovials and place them in their appropriate freezer box at -70°C. Allow "Mr. Frosty" to warm up to room temperature before using him again.

Because it may not always to be possible to have all the day's

cell samples ready to freeze at the same time, the blood laboratory will provide each site with three "Mr. Frosty" containers. Use one container per participant, if samples are processed at different times during the day.

6.7 Processing blind duplicate samples

6.71 A blind duplicate sample for quality control will be collected from 20% of participants (5% for each of four tube types). *One* of the following tube types will be drawn from the selected participant. Tube type will be based on a check digit in the participant's ID number.

- 5 mL EDTA (B-D# 366452) *or*
- 5 mL serum (B-D# 366534) *or*
- 4.5 mL citrate (B-D# 366415) *or*
- 5 mL SCAT-I (provided by CBAL)

6.72 Collect the blind duplicate tube (#9) after the regular tubes are filled.

6.73 Centrifuge EDTA, serum, citrate, and SCAT-I tubes according to the instructions in 6.3, above.

6.74 Aliquot blind duplicate samples based on the table below:

Tube type	Cryovial color	# of aliquots	Volume
EDTA	White	2	1.0 mL
Serum	Red	4	0.5 mL
Citrate	Blue	4	0.5 mL
SCAT-I	Yellow	4	0.5 mL

6.75 Label cryovials with a quality control (QC) number. This QC number will be matched to the participant ID number.

6.76 After aliquoting, immediately freeze cryovials at -70°C in an upright position.

6.77 Place blind duplicate samples (from multiple participants) in a freezer box with and 10 x 10 grid. Ship this box, according to instructions listed in section 7.3, below, to the University of Vermont (address in section 7.4, below), one week after shipping the original samples (1–62). (Delayed shipping prevents the laboratory from matching the blind duplicate samples with the participant.) When shipping, include the completed Blind Duplicate Shipping Log in lieu of the Processing Form.

6.8 Special Circumstances

6.81 If centrifugation cannot be performed within 30 minutes of collection, process specimens *as soon as possible* after that time. Record the time of collection and centrifugation on the Processing Form. Maintain the EDTA, citrate, and SCAT-I tubes on wet ice until centrifugation.

6.82 If serum and plasma cryovials cannot be frozen at -70° C within 10 minutes of aliquoting, do it *as soon as possible* after that. They may be *temporarily* placed on dry ice (preferred) or stored at -20° C until transfer to -70° C or below is possible.

6.83 If blood collection is incomplete, prioritize the processing of cryovials using the following table:

Tube	Cryovials to Be Filled
EDTA (10 mL)	1–8 and 18 (packed cells for DNA)
Serum (10 mL)	28–35
CP tube (8 mL)	45–52 and 61
SCAT-I (5 mL)	20–23 and cells into 18
Citrate (4.5 mL)	24–27 and cells into 18
EDTA (10 mL)	9–17 and191 (packed cells with dextran)
Serum (10 mL)	36–44
CP tube (8 mL)	53–05 and 62

6.9 Processing Completion

6.91 The Phlebotomy Form and MESA Processing Forms are kept in a temporary file. The Phlebotomy Form will be scanned and the information electronically sent to the Coordinating Center and the CBAL. Enclose copies of the Processing Forms with each shipment of samples to the CBAL. Upon receipt at CBAL, forms and samples are examined for monitoring and quality control purposes.

6.92 Completed, *frozen* cryovials from each participant are packed into one freezer box. A participant label is attached to the front cover of the freezer box. Frozen 4 mL and 10 mL urine tubes #s 63–65 are placed flat in remaining space in freezer box just prior to packing for shipment to Vermont. Refrigerated cryovials #18 and #19 will be shipped separately to the University of Minnesota.

6.93 Freezer Box Diagram for shipping *frozen* samples to CBAL.

Pt 1 Cryo 1	Cryo 11	Cryo 23	Cryo 33	Cryo 43	Cryo 53				

Cryo 2	Cryo 12	Cryo 24	Cryo 34	Cryo 44	Cryo 54				
Cryo 3	Cryo 13	Cryo 25	Cryo 35	Cryo 45	Cryo 55				
Cryo 4	Cryo 14	Cryo 26	Cryo 36	Cryo 46	Cryo 56				
Cryo 5	Cryo 15	Cryo 27	Cryo 37	Cryo 47	Cryo 57				
Cryo 6	Cryo 16	Cryo 28	Cryo 38	Cryo 48	Cryo 58				
Cryo 7	**Cryo 17**	Cryo 29	Cryo 39	Cryo 49	Cryo 59				
Cryo 8	**Cryo 20**	Cryo 30	Cryo 40	Cryo 50	Cryo 60				
Cryo 9	Cryo 21	Cryo 31	Cryo 41	Cryo 51	Cryo 61				
Cryo 10	Cryo 22	Cryo 32	Cryo 42	Cryo 52	Cryo 62				

6.94 Wipe down all work areas with 10% bleach solution or approved biohazard disinfectant.

6.95 Label and arrange cryovials in their proper racks for the next day's blood processing.

7. Shipping Blood Samples

7.1 **General Instructions.** Blood samples may be shipped *only* on Mondays and Tuesdays to the CBAL or the University of Minnesota by an overnight carrier (Federal Express is preferred). Samples must be shipped on a pre-arranged schedule, which allows the laboratory to stagger the arrival of samples for easier processing.

7.2 **Packaging.** Sample shipping checklist:

- Frozen MESA samples in pre-labeled sample
- Styrofoam mailing containers (2) with outer cardboard sleeves
- Rubber bands for freezer boxes
- Ziploc plastic bags for freezer boxes
- Absorbent material (e.g., paper towels, newspaper)
- Packaging tape
- Dry ice (~10 pounds per mailing container)
- Ice gel packs
- Mailing labels (provided by carrier)
- Dry ice labels
- Competed Processing Form
- Competed Shipping Form

7.3 **Procedure.** These shipping protocols follows procedures mandated by

the International Air Transport Association's Dangerous Goods Regulations-Packaging Instructions 650 and 904.

7.31 For *frozen* shipment to the University of Vermont:

1. Line Styrofoam mailer(s) with absorbent material.

2. Place approximately ½ the dry ice (about 5 pounds) on the bottom of the mailer.

3. Place another layer of absorbent material on top of the dry ice, so that it will be between the dry ice and the freezer boxes containing the samples.

4. Collect the freezer boxes containing samples to be shipped and check the sample ID numbers against the Processing Form for that shipment.

5. Put a rubber band around each cardboard freezer box containing samples before enclosing each box in a Ziploc plastic bag. Carefully place these bagged boxes into the mailer. The rubber band helps prevent cryovial spill; the Ziploc bag and absorbent material are required by commercial carriers.

6. Place another layer of absorbent material on top of the sample freezer boxes.

7. Place the remaining dry ice on top of this last layer of absorbent material.

8. Seal the top of the Styrofoam mailer with tape and then place the mailer in the outer cardboard sleeve.

9. Place the Processing Form, listing all samples included in that particular Styrofoam mailer, on the top of the Styrofoam mailer before the outer sleeve is securely taped closed.

10. Fill out the shipping log, including the carrier airbill numbers, and fax to the University of Vermont at (802) 656-8965.

11. Affix shipping label(s). Place the entire box in the refrigerator, if pickup will not bee immediate. (Samples should not be on dry ice for >24 hours.)

7.32 For *refrigerated* shipment to the University of Minnesota:

1. Line Styrofoam mailer(s) with absorbent material.

2. Place two frozen gel ice pack on the bottom of the mailer.

3. Place another layer of absorbent material on top of the gel ice packs. Do not let the tubes come into direct contact with the gel ice packs.

4. Collect the freezer boxes that contain tubes #18 and #19 and check the sample ID numbers against the Processing Forms for that shipment.

5. Put a rubber band around each cardboard freezer box containing samples before enclosing each box in a Ziploc plastic bag. Carefully place these bagged boxes into the mailer. The rubber band helps prevent tube spill; the Ziploc bag and absorbent material are required by commercial carriers.

6. Place another layer of absorbent material on top of the sample freezer boxes.

7. Place another two or three frozen gel ice pack on top of this last layer of absorbent material.

8. Seal the top of the Styrofoam container with tape, place it in the outer cardboard sleeve, and then seal the sleeve with tape.

9. Fill out the shipping log, including the carrier airbill numbers, and fax to the University of Minnesota at (612) 273-3489.

10. Affix the shipping label. Place the entire box in the refrigerator, if pickup will not bee immediate.

7.4 Mailing Addresses:

[Removed for LAD purposes]

8. Quality Assurance (QA)

8.1 Overview of Field Center Monitoring

QA monitoring of the blood collection and processing protocols is important for the identification of any deviations from the standardized methods. Differences in the way blood samples are collected or processed have the potential to create statistically significant

differences in assay results. In order to prevent any sample-associated problems, the CBAL has designed a system for monitoring the quality of blood collection and processing in each field center. Monitoring will assure that any systemic or random problems are identified and corrected. Components of the program include:

- CBAL training course and certification process for each field center technician;
- maintenance of equipment check logs at each field center;
- field center supervisor checklist;
- Phlebotomy Form and MESA Processing Form review by the CBAL; and
- analysis of problems associated with the phlebotomy.

8.2 Field Center Technician Training and Certification

Standardization of venipuncture and blood processing procedures helps to ensure the quality of the blood samples and subsequent data analysis. CBAL will conduct a one-time training session on blood collection and processing of the MESA samples, which will be held at the MESA meeting at Northwestern University in April 2000. The training session will present information relating to the collection of the blood sample (e.g., infection control, safety precautions and OSHA regulations, equipment handling, venipuncture procedure, and possible venipuncture problems) and proper processing procedures for the varied array of draw tubes (e.g., centrifugation, temperature requirements, and aliquoting).

8.21 Field center technician requirements. Field center technicians who will be performing blood collection must have prior clinical phlebotomy experience. They must read the MESA Manual of Operations before attending the CBAL training session. Certification in MESA blood collection and processing is required.

8.22 Field center technician certification. Field center technicians who attend the LCBR training session and successfully complete both the written and practical examinations will be certified in MESA blood collection and processing. (Completed written exams will be corrected and kept on file at the CBAL.) Once fully certified, a technician is qualified to certify other technicians in the complete or partial process. The three steps required for certification are:

- Successful completion of written exam (prepared by CBAL)
- Successful completion of practical exam (using the

Certification Form in this Manual).
- Observation by certified personnel of complete phlebotomy/processing procedure on a volunteer.

8.3 Field Center Equipment Records

Each field center must maintain daily and monthly records of equipment performance. Personnel should perform daily temperature checks on refrigerators, freezers, and refrigerated centrifuges and maintain equipment temperature logs for future reference. These equipment records help to identify problems with sample quality in the aliquoting and local storage steps.

8.4 Field Center Supervisor Checklist

The field center supervisor is required to periodically observe MESA technicians in the performance of the phlebotomy and processing procedures, recording their observations on the checklist. Completed supervisor checklists will be sent to the CBAL for monitoring purposes.

9. Field Center Forms

- MESA Phlebotomy/Processing Form.
- MESA Shipping Log
- MESA Blind Duplicate Shipping Log
- MESA Field Center Supervisor Checklist
- MESA Field Center Technician Certification Examinations
- MESA Equipment Temperature Logs

10. Reagent Descriptions

10.1 Remember to label reagents carefully. Include the date made (PBS) or thawed (freezing media) on the stock solutions.

10.2 **Phosphate Buffered Saline (PBS)**

Phosphate tablets are combined with 200 mL of de-ionized water to make the PBS that is used in cell centrifugation. To make PBS, pour 200 mL de-ionized water into a graduated cylinder and add one phosphate tablet. Pour into a reagent bottle, allowing approximately 30 minutes for the tablet to dissolve. This bottle is your stock solution of PBS, which may be refrigerated for no more than five days. After five days discard unused PBS (it is safe to pour down the drain). When PBS is needed, pour off what is needed for the day into the working container and allow it to warm up to room temperature before using

(approximately 30 minutes). You will need approximately 28–30 mL per participant.

10.3 Freezing Media

10.31 Freezing solutions A and B are specially-prepared preservatives for the white cells. The solutions will be shipped frozen, directly from the manufacturer (BioSource International, Inc., BioFluids Division, Rockville, MD). Each bottle contains 50 mL. Store at -20° C until ready for use. Gently swirl the thawed media to ensure thorough re-suspension of the product. Divide the content of each 50 mL bottle into two 25 mL bottles (or tubes) with screw caps. Clearly label each new bottle with the media type (A or B), the lot number (written on the 50 mL bottle), and date thawed and aliquoted. These 25 mL bottles will be your "stock" solution.

10.32 Promptly re-freeze all 25 mL bottles (or tubes) at -20°C, except one each of Freezing Media A and B. Store these two bottles in the refrigerator, where they will remain stable for approximately two weeks.

10.33 The Freezing Media is photosensitive and will deteriorate with prolonged exposure to light. Do not store in a lighted cold room, cooler, etc. The day's "working" solution containers can be wrapped in foil if other light protective measures are not available.

10.34 One mL each of Freezing Media A & B will be needed to process the two CP tubes for each participant. Each morning remove the volume of freezing media needed to process that day's samples from the refrigerated 25 mL bottle. Keep the day's "working" solutions for both A and B on ice or in the refrigerator. According to the manufacturer, it is important to swirl the freezing media before pipetting.

10.35 At the end of the day, any unused portion of the Freezing Media from the day's "working" solution container may be refrigerated. Do not add the unused portion back to the current stock bottle. Do not use a "working" solution for more than two days.

10.36 As the volume of the refrigerated "stock" solution starts to get low, remember to allow time to thaw a new 25 mL aliquoted bottle (or a new 50 mL original bottle) overnight in the refrigerator. If there is any unused Freezing Media remaining in the 25mL bottle that has reached it's maximum two-week

refrigerated storage life, discard it.

10.4 **ACD Solution**

10.41 Dextran preservative solution, which is provided by CBAL, is added to the EDTA packed red blood cells in cryovial 19. The solution, which must be refrigerated, will last for at least one year.

10.42 Transfer the approximate volume needed (3-4 mL per participant) for the day's work into a separate container. Keep this "working" solution on ice or in the refrigerator during the day.